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Altered membrane ionic permeability in a rat model of chronic renal failure

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Altered membrane ionic permeability in a rat model of chronic renal failure. Acute elevations in intracellular adenosine 3′,5′-cyclic monophosphate (cAMP) concentrations are known to increase ionic chloride permeability in diverse tissues. To determine if chronic endogenous increases in cAMP are associated with sustained alterations in membrane ionic permeabilities, renal cortical brush border membrane vesicles (BBMV) were prepared and red blood cells were harvested in a model of chronic renal failure, the 75% nephrectomized rat. Relative ionic permeabilities were determined using the potential-sensitive fluorescent probe 3,3′-dipropylthiadicarbocyanine iodide [diS-C3-(5)]. These studies demonstrate that renal cortical homogenate and RBC cAMP concentrations are increased in chronic renal failure animals. In the same animals relative ionic chloride permeability (PCl/PK) was significantly increased in renal cortical BBMV and RBC ghosts: PNa/PK was not affected. This selective change in permeability results in a significant increase in PCl/PNa and hyperpolarization of BBMV of sufficient magnitude to stimulate Na^+-dependent glutamine transport. The change in glutamine uptake was not consequent to an alteration in the kinetics of glutamine transport or delayed dissipation of the inward Na^+ gradient. Renal hypertrophy *per se* did not effect renal homogenate cAMP concentration or relative ionic permeability of renal cortical BBMV prepared from kidneys of uninephrectomized animals fed a 40% protein diet. These studies demonstrate that relative ionic chloride permeability and tissue [cAMP] are chronically increased in diverse cells (renal proximal tubule and RBCs) in a rat model of renal failure. These findings suggest that membrane ionic permeability may be altered and electrogenic transport secondarily perturbed in renal failure in association with hormonally-induced chronic elevations of intracellular cAMP concentrations.

Previous studies have demonstrated that acute elevations in the intracellular second messenger cAMP can induce and/or regulate conductive chloride pathways and thereby alter the membrane potential (P.D.) [1–3] in various epithelia such as kidney [1, 4, 5], trachea [6, 7], gallbladder [3, 8], and shark rectal gland [2]. This messenger has also been reported to modulate ionic chloride permeability in some non-polarized cells, the normal human red blood cell [9] and the cultured lymphocyte [10]. While these observations were made during pharmacologic manipulations that acutely elevated intracellular cAMP concentrations, similar changes have also been detected in membranes of cells in which cAMP is endogenously and chronically increased: relative ionic chloride permeability of RBC ghosts and RBC cAMP concentrations are both increased in untreated uremic humans; after treatment with peritoneal dialysis both are indistinguishable from those in normal volunteers [9]. Based on the fact that renal failure is accompanied by elevated plasma concentrations of several hormones that activate adenylate cyclase (PTH, norepinephrine, glucagon, and ADH) [11–13], it was postulated that an increase in relative ionic chloride permeability might not be confined to the red cell, but rather might represent a more global change in membrane permeability in chronic renal failure. Since intracellular chloride activity is at or above electrochemical equilibrium in diverse tissues [1, 7, 8, 14, 15], a selective increase in relative ionic chloride permeability would depolarize cells [1, 3, 5]. If such were the case, the resultant perturbations in cell function (that is, transport and electrical signaling) could be responsible, at least in part, for a number of systemic manifestations of renal failure.

The present studies were designed to determine whether an increase in membrane ionic chloride permeability is confined to human red cell membranes or whether such an increase occurs in other cells in renal failure. The 75% nephrectomized rat was used as a model of renal failure. These studies demonstrate that renal failure in the rat is accompanied by increases in renal cortical cAMP concentration and relative ionic chloride permeability of renal cortical brush border membranes. Of note, these changes were not confined to renal cortical membranes. As in the human [9], red blood cell cAMP concentrations and the ionic chloride permeability of RBC membranes were significantly increased in renal failure in the rat. The demonstration of qualitatively similar findings in membranes of both polarized and non-polarized cells suggests that membrane ionic permeability may be significantly altered in a number of tissues in renal failure in association with hormonally induced increases in intracellular concentrations of cAMP.

Methods

Male Sprague-Dawley rats were divided into sham operated, uninephrectomized and 75% nephrectomized groups. All animals were anesthetized by an intraperitoneal injection of pentobarbital 45 mg/kg body wt (Sigma Chemical Co., St. Louis, Missouri, USA). Thereafter, kidneys were exposed by blunt dissection via bilateral flank incisions. The right kidney was excised in uni-nephrectomized and 75% nephrectomized animals; 75% nephrectomy was produced by further ligation of the

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dorsal branch of the left renal artery under microscopic observation [16]. Postoperatively animals were maintained in metabolic cages. Sham operated and 75% nephrectomy animals were pair fed 20 g/day of a 24% protein rat chow (No. 5010, Ralston Purina Co., St. Louis, Missouri, USA); water was allowed *ad libitum*. Uninephrectomized animals were fed 20 g/day of a 40% protein rat chow (No. TD86211, Teklad, Madison, Wisconsin, USA). Two to three months post-surgery urine was collected in thymol for the determination of creatinine and cAMP concentrations. On the following day animals were anesthetized and the kidneys were harvested from six to eight uninephrectomized or 75% nephrectomized animals, and four to five sham operated rats (controls). Heparinized blood was then collected into tubes containing 3-isobutyl-l-methylxanthine (IBMX, Sigma), a phosphodiesterase inhibitor, at a final concentration of 1 mM for preparation of red blood cell ghosts and the determination of red blood cell cAMP concentration. Urea nitrogen and creatinine concentrations were determined in aliquots of blood obtained from individual animals [17, 18].

Preparation of membrane vesicles

BBMV were prepared by magnesium aggregation as previously reported [4, 19]. In brief, kidneys were removed and immediately placed in ice-cold 300 mM mannitol, 10 mM Tris-HEPES, pH 7.4 (MTH). After removal of the renal capsule, kidneys were weighed and cortical slices were obtained, weighed, placed in fresh ice-cold buffered mannitol (1 ml/g) and minced. The homogenate was diluted to a final volume of 10 ml/g cortex with the same buffered mannitol containing 1 mM IBMX. Following the addition of $MgCl_2$ (final concentration 10 mM) the homogenate was stirred gently on ice for 10 minutes. Thereafter, three low speed centrifugations were alternated with three high speed centrifugations. The final pellet was resuspended by vortexing with a volume of 10 mM $MgCl_2$, 300 mM MTH estimated to yield a protein concentration of 10 to 15 mg/ml. Thereafter the BBMV were preloaded in KCl by preincubation in 100 mM KCl, 100 mM MTH, pH 7.4 at 22°C for three hours and then stored on ice until study.

To assess the purity of the vesicle preparations, enzyme assays were performed at 37°C on both whole homogenate and isolated brush border fractions. Alkaline phosphatase, measured with a test kit (Sigma Chemical Co.) was used as a marker for the brush border membrane and K^+-dependent p-nitrophenyl phosphatase [20] was employed as a basolateral membrane marker. The specific activities of alkaline phosphatase and Na^+/K^+ ATPase in the 75% nephrectomized membranes, 860 ± 85 and 37.5 ± 13.5 nmol/min per mg protein, respectively, were not different from that of control preparations, 824 ± 61 and 37.4 ± 3.0, respectively. The enrichments of alkaline phosphatase in the 75% nephrectomy (9.9 ± 0.9) and control (8.9 ± 0.6) membranes were not different. Furthermore, there was no significant difference in the de-enrichment of Na^+/K^+ ATPase in 75% nephrectomy (0.67 ± 0.18) and control vesicle preparations (0.86 ± 0.12). The protein concentration of cortical homogenates, BBMV, and ghosts were determined by the method of Lowry et al [21] using bovine serum albumin as the standard.

Preparation of red blood cell ghosts

Ghosts were prepared using a previously reported [9, 22] modification of the hypotonic lysis method of Bodemann and Passow [23] and Steck [24]. Seven hundred and fifty microliters of ghosts were resuspended in 40 ml of 100 mM KCl or 100 mM NaCl, 50 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), buffered to pH 6.5 with 5 mM KOH or NaOH, respectively, and incubated at 37°C for 90 minutes to reseal them. Thereafter, the resealed ghosts were centrifuged at 30,000 g × 15 minutes. The pellet of ghosts was washed and centrifuged two additional times in ice cold solutions identical to those in which they were sealed. The pelleted KCl or NaCl preloaded ghosts were stored on ice until study.

Estimation of membrane KCl permeability and relative ionic permeabilities

$[KCl]_{in}$ and relative ionic permeabilities of KCl preloaded BBMV and ghosts were assessed with the positively charged P.D. sensitive fluorescent probe 3,3'-dipropylthiadicarbocyanine iodide, [diS-C_3-(5)], utilizing techniques identical to those previously described [9, 19]. In brief, initial fluorescence was recorded following addition of aliquots (100 μg protein) of membrane vesicles or ghosts to media containing 3 μM diS-C_3-(5), 0 to 100 mM KCl and either 100 mM MTH, pH 7.4 (BBMV) or 50 mM K-HEPES, pH 6.5 (ghosts). The $[K^+]_{out}$ was varied from 0 to 100 mM by replacing KCl with equimolar concentrations of choline Cl. When fluorescence stabilized (40 sec), valinomycin (3 μM) was added and fluorescence was monitored. Since valinomycin produces no change in fluorescence when $[K^+]_{in} = [K^+]_{out}$ [4, 9, 19], $[KCl]_{in}$ was calculated by determining the $[K^+]_{out}$ at the intersection of the regression lines of the fluorescence recorded at each medium $[K^+]$, in the absence and presence of valinomycin. The prevalinomycin regression line was fitted to the Goldman-Hodgkin-Katz constant field equation [4, 9, 19]; the postvalinomycin line was fitted to the Nernst equation[1] [4, 9, 19]. After $[KCl]_{in}$ was determined, fluorescence was converted to membrane potential (P.D.) in millivolts [4, 9, 19]. The ionic permeability of Cl^- relative to potassium (PCl/PK) was then calculated using the Goldman equation [4, 9, 19]. By substituting 100 mM NaCl or RbCl for the 100 mM KCl in the medium and converting initial fluorescence in these media to P.D. (in the absence of valinomycin), PNa/PK and PRb/PK were calculated using the Goldman equation.

Sodium uptake experiments

The uptake of ^{22}Na (NEN DuPont Research Products) was determined by addition of 10 μl of unloaded vesicles prepared from control or renal failure homogenates to 40 μl of medium. At specified times after incubation in 100 mM $^{22}NaCl$, 100 mM MTH at 22°C, uptake was stopped by the rapid addition of 3 ml of ice-cold rinse solution (150 mM KCl, 10 mM Tris-HEPES, pH 7.4). Vesicles were then immediately separated from their media by filtration through 0.45 μm filters (HATF, Millipore,

[1] In cells in which gradients exist for Na as well as K and Cl, it would be necessary to use the Goldman rather than the Nernst equation. The assumption that valinomycin produces no change in P.D. when $K_{in} = K_{out}$ can therefore only be applied to the electrolyte conditions defined in these studies.

Table 1. Functional parameters of control, uninephrectomized, and remnant kidney animals

	Control	P^a	Uninephrectomy	P^b	Remnant	P^c
Body wt *g*	434 ± 15	<0.005	367 ± 11	<0.05	392 ± 6	<0.005
Kidney wt *g*	1.39 ± 0.03	<0.0001	1.90 ± 0.04	NS	1.85 ± 0.05	<0.0001
GFR/kidney *ml/min*	1.02 ± 0.08	NS	1.18 ± 0.07	<0.0001	0.67 ± 0.05	<0.0001
BUN *mg/dl*	14.3 ± 1.4	<0.0001	32.3 ± 2.7	NS	27.5 ± 0.4	<0.0005
Creatinine *mg/dl*	0.65 ± 0.07	NS	0.65 ± 0.02	<0.0001	1.04 ± 0.07	<0.005
$U_{Na}V$ *μEq/24 hr*	2197 ± 156				2360 ± 112	NS
U_KV *μEq/24 hr*	3945 ± 185				4170 ± 245	NS

Values represent mean ± SE. *P* indicates statistical significance between groups: [a] control vs. uninephrectomy, [b] uninephrectomy vs. remnant, and [c] control vs. remnant.

Bedford, Massachusetts, USA). The incubation tubes and filters were rinsed with an additional 6 ml of rinse solution, the filters were rapidly removed from the individual filtration manifolds, placed in scintillation vials, dried, diluted in liquid scintillant and counted. Sodium uptake in nanomoles/mg protein was determined from the CPM of each filter, the specific activity of sodium, and the protein concentration of the vesicle preparation.

Sodium-dependent glutamine uptake

The uptake of L-[^{3}H] glutamine (New England Nuclear, Boston, Massachusetts, USA) was measured by addition of 10 μl of membrane vesicles (devoid of intravesicular electrolytes) prepared from control or renal failure homogenates to 40 μl of media containing a final concentration of 50 μM [^{3}H]glutamine, 100 mM NaCl, 100 mM MTH at 22°C. At specified times, uptake was stopped by the rapid addition of 3 ml of ice-cold rinse solution (150 mM NaCl, 10 mM Tris-HEPES, pH 7.4). Subsequent steps were as described above in the sodium uptake studies. Uptake in pmol/mg protein was determined from the DPM on each filter, the specific activity of glutamine, and the protein concentration of the vesicle preparation. The kinetics of Na^+-dependent glutamine transport were also assessed in vesicles prepared from control and renal failure homogenates. Because preliminary studies indicated that glutamine uptake was linear for at least 15 seconds, 10 second uptakes were performed by methods described above in media containing 0.025 to 8.0 mM [^{3}H] glutamine, 100 mM NaCl or 100 mM KCl, 100 mM MTH and 80 μM carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP, Sigma) to clamp the membrane potential [4]. Na^+-dependent glutamine uptake was calculated as the difference in uptake in the presence of NaCl and KCl. The apparent affinity (K_m) and maximum velocity (V_{max}) of the high and low affinity sites for glutamine transport were calculated from Michaelis Menten plots of the 10 second uptakes using non-linear regression analysis (Enzfitter, Elsevier-BIOSOFT, Cambridge, UK).

Measurement of cAMP levels

cAMP concentrations were determined in aliquots of plasma, renal cortical homogenates and RBCs using a radioisotopic test kit (Amersham, Arlington Heights, Illinois, USA). Plasma was assayed directly. Renal cortical homogenates were prepared for cAMP assay by adding 0.5 ml of the homogenate to 1.0 ml boiling H_2O for three minutes [25]. After cooling on ice and centrifugation the supernatant was assayed for cAMP content. RBC cAMP was extracted by adding 0.5 ml of a 50% hematocrit of RBCs to 1.5 ml boiling H_2O. The mixture was boiled for an additional three minutes [25], cooled on ice and centrifuged. An aliquot of the supernatant was lyophilized to dryness and then reconstituted to 1/5th its original volume. cAMP concentrations in plasma, cortical homogenates and RBCs are expressed as pm/ml plasma, pm/mg homogenate protein and pm/ml packed RBC, respectively.

Statistics

In each experiment, studies were performed and analyses made on triplicate or quadruplicate samples of vesicles or ghosts prepared on the day of the experiment. The mean of the triplicate or quadruplicate determinations provided one value in the calculation of the mean of all experiments. All data are expressed as the mean ± SE. Unpaired analysis and Student's *t*-test were used to determine statistical significance.

Results

Uninephrectomized animals

Significant renal hypertrophy occurs in the remnant kidney following 75% nephrectomy [16, 26]. Experiments were therefore performed to evaluate whether hypertrophy *per se* was associated with changes in the relative ionic permeabilities of brush border membranes or renal cortical cAMP concentrations. Uninephrectomized animals on a diet containing 40% protein developed substantial renal hypertrophy. As demonstrated in Table 1, kidney weight of uninephrectomized animals on a high protein diet was significantly greater than that of age matched, sham operated controls (1.90 ± 0.04 vs. 1.39 ± 0.03 g). However, glomerular filtration rate (GFR) as estimated from creatinine clearance was comparable to the single kidney GFR in control animals. Blood urea nitrogen (BUN) concentration was elevated in uninephrectomized animals as a consequence of the increase in dietary protein. Despite the significant hypertrophy, no significant differences were detected in either the relative ionic permeabilities of BBMV (Table 2) or renal cortical homogenate cAMP concentrations of control and uninephrectomized animals (5.62 ± 1.79, $N = 4$ vs. 5.76 ± 1.42 pm/mg homogenate protein, respectively, $N = 4$).

75% Nephrectomized animals

As summarized in Table 1, the body weight of remnant kidney animals was reduced compared to age-matched, pair-fed, sham-operated two-kidney controls. The weight of remnant kidneys was significantly greater than that of the controls. Of interest, the weight of the remnant kidney was not different

Table 2. $[K^+]_{in}$ and relative ionic permeabilities in BBMV of control and uninephrectomized animals

	Control	Uninephrectomy	*P*
$[K^+]_{in}$	65.4 ± 1.24	70.7 ± 1.49	NS
PCl/PK	1.23 ± 0.13	1.30 ± 0.10	NS
PNa/PK	0.79 ± 0.07	0.74 ± 0.06	NS
PCl/PNa	1.67 ± 0.31	1.79 ± 0.14	NS
PRb/PK	1.02 ± 0.09	0.97 ± 0.25	NS

$[K^+]_{in}$ is the intravesicular potassium concentration (mM). PCl, PK, PNa, and PRb are the ionic permeabilities of chloride, potassium, sodium, and rubidium, respectively. Values represent means ± SE of data from five experiments. *P* represents statistical significance.

Table 3. cAMP concentrations and urinary cAMP excretion in control and remnant kidney animals

	Control	Remnant	% Control	*P*
Kidney *pm/mg prot*	4.30 ± 0.58	5.25 ± 0.61	126 ± 7	<0.005
Urinary excretion *μm/24 hr*	4.64 ± 0.71	6.21 ± 0.57	133 ± 10	<0.05
RBC *pm/ml RBC*	46.3 ± 9.6	57.3 ± 7.4	149 ± 20	<0.05
Plasma *pm/ml*	25.8 ± 5.8	23.2 ± 8.4	83 ± 18	NS

Values are means ± SE. *P* indicates statistical significance between control and renal failure samples after normalizing control values to 100%.

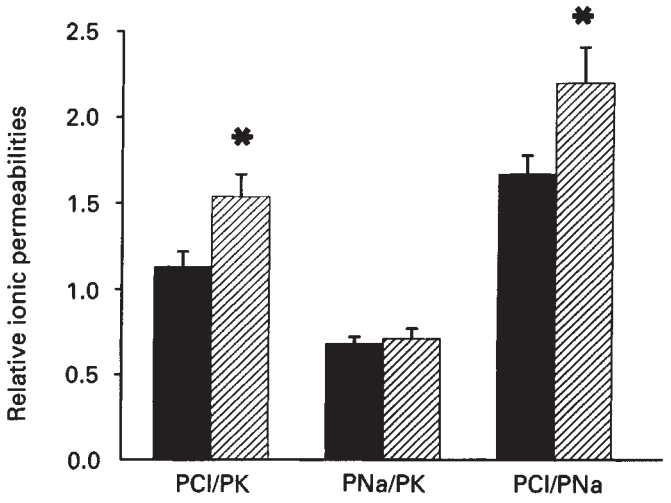

Fig. 1. *Relative ionic permeabilities of BBMV prepared from control and 75% nephrectomized animals.* PCl/PK and PNa/PK are ionic permeabilities of chloride and sodium, respectively, relative to potassium, and PCl/PNa is ionic permeability of chloride relative to sodium. Solid and hatched bars represent mean data from control and 75% nephrectomized animals, respectively. * indicates statistical significance between groups ($P < 0.05$) in 7 experiments. Intravesicular potassium concentration $[K^+]_{in}$ (67.8 ± 1.3 vs. 69.2 ± 0.86 mM) and the relative ionic permeability of rubidium to potassium, PRb/PK (1.00 ± 0.07 vs. 1.14 ± 0.13) were not statistically different in control and 75% nephrectomized animals.

from that of uninephrectomized animals. However, since approximately one half of the remnant kidney was surgically infarcted, it is evident that the magnitude of hypertrophy was substantially greater in individual nephrons of the remnant kidney. Renal function was significantly reduced in the remnant kidney: BUN was increased and GFR (estimated from the creatinine clearance) was decreased 33% relative to the single kidney GFR of the control. In contrast to the uninephrectomized animals, renal cortical homogenate cAMP concentration and urinary cAMP excretion were significantly increased in remnant kidney animals (Table 3).

Brush border membrane vesicles

The effects of renal failure on the permeabilities of Cl^-, Na^+ and Rb^+ relative to that of potassium in BBMV prepared from control and remnant kidney animals are indicated in Figure 1. PCl/PK was increased in each study in vesicles prepared from 75% nephrectomized animals: the mean value increased significantly by 36%. In contrast, PNa/PK and PRb/PK were not significantly affected in remnant kidney vesicles. As a consequence of the selective increase in PCl/PK there was a significant increase in PCl/PNa. Although the increase in relative chloride permeability could result from an increase in ionic chloride permeability and/or a proportional fall in both potassium and sodium permeability, the latter possibility seems unlikely since ^{22}Na uptake was not reduced in vesicles of remnant kidney animals (see below). Accordingly, the increase in PCl/PK and PCl/PNa in BBMV of the renal failure animals appears primarily to represent an absolute increase in PCl.

As a consequence of the increase in PCl/PNa in renal failure membranes, the Goldman equation predicts that an inwardly directed 100 mM NaCl gradient would result in a greater hyperpolarization of vesicles from renal failure animals compared to controls. Based on the measured PCl/PNa in renal failure and control membranes, imposition of an inward 100 mM NaCl gradient in previously unloaded vesicles results in a significant increase in the calculated inside negative membrane potential in remnant kidney BBMV (−19.9 ± 2.3 mV) compared to the controls (−13.3 ± 2.6 mV; $P < 0.05$; Fig. 2). Insofar as this more negative membrane potential should stimulate electropositive transport processes, electropositive, Na^+-dependent glutamine uptake [27] was compared in BBMV of control and remnant kidneys. In four studies, Na^+-dependent glutamine uptake was significantly increased at one minute (19.6 ± 6.1%) in BBMV of renal failure animals, whereas the equilibrium uptakes (at 2 hr) were not significantly different from control (Fig. 3). Of note, the percentage increase in sodium-dependent glutamine uptake at one minute is entirely consistent with the previously reported theoretical and observed fractional increment in sodium-dependent glucose uptake induced by a 6 to 7 mV change in membrane potential, when the P.D. was varied between −10 and −20 mV [28].

To determine whether the stimulation of Na^+-dependent glutamine transport resulted from modification of the glutamine transporter *per se* rather than the change in ionic permeabilities, the kinetics of the high and low affinity sites of this transporter were studied. To obviate the effect of membrane potential, kinetic parameters were determined in the presence of the protonophore FCCP to "clamp" the membrane potential. The K_m and V_{max} of the high and low affinity sites of the glutamine transporter were not affected by renal failure. In two studies the mean K_m and V_{max} of the high affinity transporter were 66 versus 57 μM and 365 versus 260 pm/mg protein/10 sec in control and renal failure BBMVs, respectively. The mean K_m and V_{max} of the low affinity transporter were 8 versus 5.8 mM

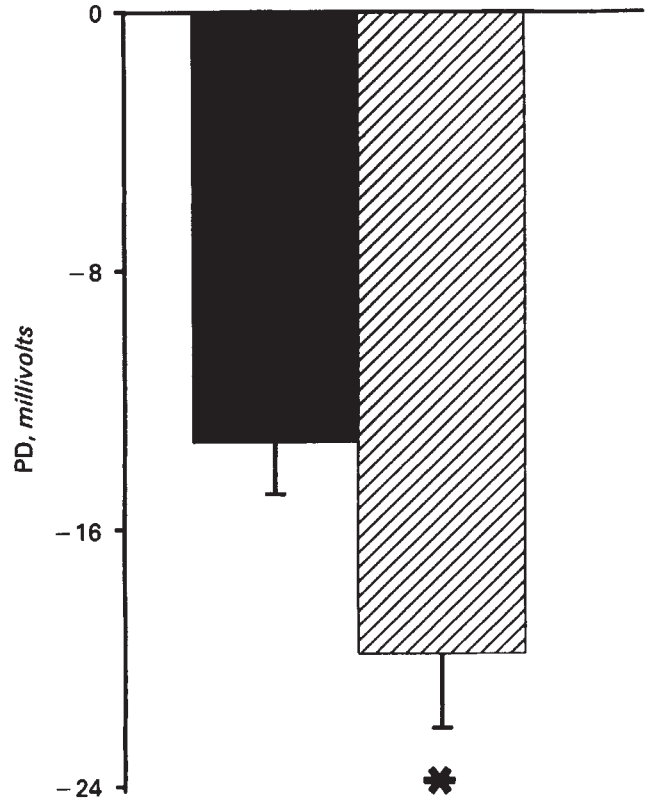

Fig. 2. *The calculated initial membrane potential (P.D.) of unloaded BBMV prepared from control and 75% nephrectomized animals in the presence of a 100 mM inwardly directed NaCl gradient.* Solid and hatched bars represent mean data from control and 75% nephrectomized animals, respectively. * indicates statistical significance between groups ($P < 0.05$) in 7 experiments.

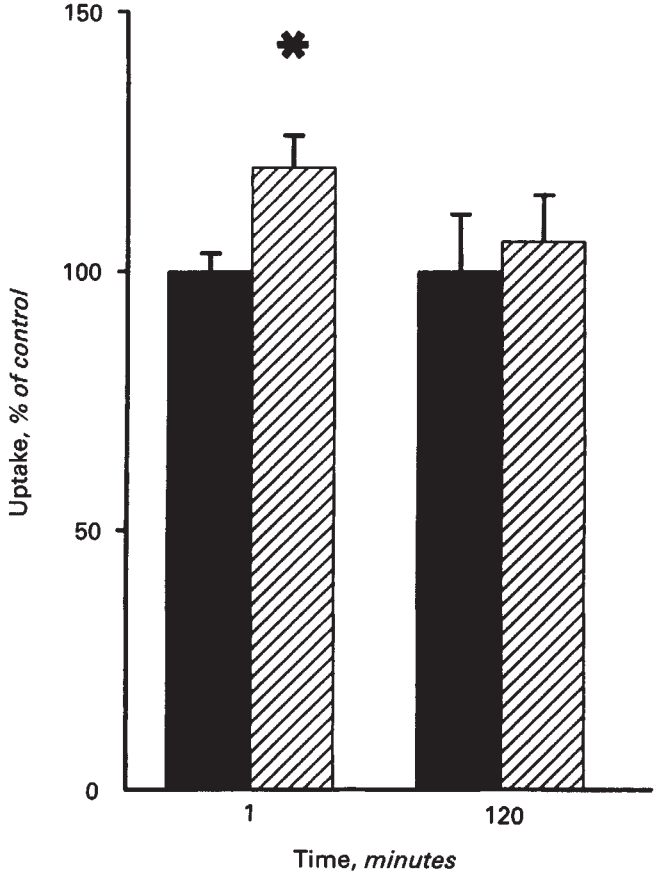

Fig. 3. *Na^+-dependent glutamine uptake in BBMV.* Solid and hatched bars represent mean data from control and 75% nephrectomized animals, respectively. * indicates statistical significance between groups ($P < 0.05$) after normalizing control values to 100% in 4 experiments.

and 14 versus 12 nmol/mg protein/10 sec in control and renal failure BBMV, respectively. Alternatively, since cAMP has been shown to inhibit Na^+-H^+ antiport activity [29], a reduction in the activity of this transporter could sustain an inward Na^+ gradient and thereby increase Na^+-dependent glutamine uptake in the renal failure animals. However, in two studies the mean $^{22}Na^+$ uptake at one minute was not reduced in BBMV of renal failure animals (60 vs. 76 pmol/mg protein at 1 min in control vs. renal failure, respectively). These cumulative observations indicate that the stimulation of Na^+-dependent glutamine uptake cannot be ascribed to a change in the kinetics of this transporter or delayed dissipation of the inward Na^+ gradient. Rather, an increased driving force consequent to the increase in PCl/PNa and resultant hyperpolarization of the intravesicular space appears responsible for the stimulation of Na^+-glutamine cotransport in BBMV of renal failure animals.

RBC ghosts

The relative ionic permeabilities of RBCs harvested from uninephrectomized animals were indistinguishable from those in sham operated controls. The effects of renal failure on relative ionic permeabilities of RBC ghosts, depicted in Figure 4, are qualitatively similar to those of brush border membranes prepared from the remnant kidney. Neither $[K^+]_{in}$ nor PNa/PK were significantly different in ghosts prepared from control and remnant kidney animals. However, in each experiment PCl/PK was significantly higher in ghosts prepared from animals in renal failure. It must be noted that while the PCl/PK of red cell ghost membranes is significantly lower than that observed in intact red blood cells [9], the mechanism of this reduction is not completely understood. Insofar as ghosts maintain KCl gradients, this effect cannot be ascribed to a failure of the ghosts to reseal. Moreover, since ionic potassium permeability is increased in RBC ghosts, presumably as a consequence of the ghosting process [30], it is possible that the lower PCl/PK in ghosts represents a rise in PK, rather than a fall in PCl. Independent of the mechanism, as RBC ghosts of renal failure and control animals were prepared simultaneously and identically, it is assumed that the same factors that are responsible for the reduction in PCl/PK relative to that of the intact red cell membrane would be operative in both populations in ghosts. In this context, any differences in ionic permeabilities between renal failure and control membranes should be consequent to factor(s) related to the renal failure and not the ghosting process.

Red blood cell cAMP concentrations were not different in uninephrectomized and sham operated controls. In contrast, red blood cell cAMP concentration was significantly higher in

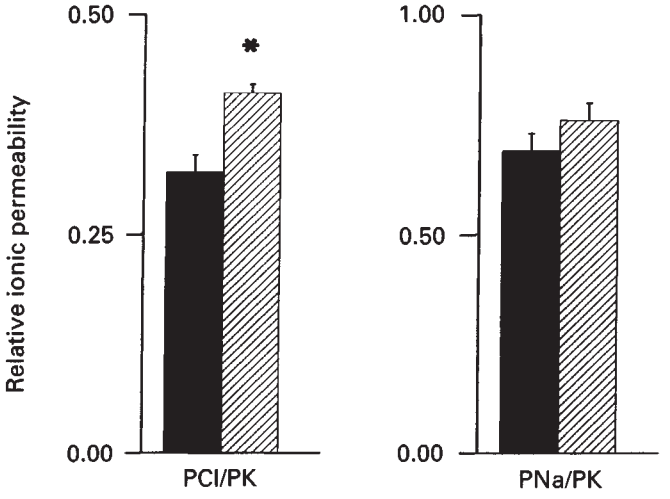

Fig. 4. *Relative ionic permeabilities of red blood cell ghosts prepared from control and 75% nephrectomized animals.* PCl/PK and PNa/PK are ionic permeabilities of chloride and sodium, respectively, relative to potassium. Solid and hatched bars represent mean data from control and 75% nephrectomized animals, respectively. * indicates statistical significance ($P < 0.05$) between groups in 7 experiments. Intravesicular potassium concentration $[K^+]_{in}$ (84.7 ± 2.7 vs. 77.2 ± 2.40 mM) was not statistically different in control and 75% nephrectomized animals.

remnant kidney animals, while plasma cAMP concentrations were not different in control and renal failure animals (Table 3). Thus, renal ablation produced qualitatively similar changes in both renal and red blood cell intracellular cAMP concentrations and relative ionic permeabilities of renal cortical and red blood cell membranes prepared from the 75% nephrectomized rat.

Discussion

There is an increasing body of evidence that indicates that the ionic permeabilities of plasma membranes of both polarized and non-polarized cells are not static, but rather that they are modulated by acute variations in the concentration of the intracellular second messenger cAMP [1–8, 10, 31–33]. The present studies provide evidence that relative ionic chloride permeability of renal proximal tubule brush border membranes is increased in renal failure in the rat (Fig. 1) and that this alteration in ionic permeability is also associated with a chronic increase in tissue cAMP concentration (Table 3). Because of the marked hypertrophy of residual nephrons in the ablation model of renal failure, consideration was given to the possibility that the changes in renal membrane ionic permeability and tissue cAMP concentration were consequent to hypertrophy rather than the renal failure. However, membranes prepared from hypertrophied nephrons of uninephrectomized, protein loaded animals failed to demonstrate alterations in either ionic permeability or tissue cAMP concentration (Table 2). While the increase in PCl/PK in renal failure might be ascribed to differences in the extent of hypertrophy in uninephrectomized and 75% nephrectomized animals, cells that do not hypertrophy in renal failure, the RBC, also demonstrated a selective increase in membrane relative ionic chloride permeability (Fig. 4) and an increase in red blood cell cAMP concentration (Table 3). The latter findings strongly implicate renal failure, and not hypertrophy, as the proximate cause of both the change in proximal tubule brush border membrane ionic permeability and tissue cAMP concentration.

Although ionic permeability to chloride was readily demonstrable in membrane vesicles of both control and renal failure animals (Fig. 1), earlier studies suggested that the proximal tubule cell membrane ionic permeability to Cl^- is exceedingly low in vivo in the basal state [34]. As a consequence, the existence of chloride channels in proximal tubule membranes has been questioned. However, recent patch clamp evidence indicates that intact proximal tubule cells do possess chloride channels whose open probability is significantly increased by hormones (such as PTH), second messengers, and protein kinases A and C [31]. The low basal activity of chloride channels in vivo [34] has recently been ascribed to the presence of a cytosolic heat resistant 0.7 to 1.5 K_d molecule that tonically inhibits chloride channel activity [35, 36]. Indeed, removal of this inhibitor unmasks chloride channel activity in renal membranes [35]. In this context, the increased relative chloride permeability of BBMV [4, 19, 37, 38] compared to that of proximal tubule cells in vivo [34–36] appears to reflect a partial disinhibition of chloride channel activity. The demonstration that the ionic chloride permeability of BBMV can be further increased in association with acute [4] and chronic endogenous elevations of renal cortical homogenate cAMP concentration (Table 3), and by hormonal or toxin induced activation of the guanine nucleotide stimulating (G_s) and inhibitory (G_i) proteins [39], suggests that the cAMP pathway participates in modulating chloride channel activity in renal membranes in vivo.

As the electronegative membrane potential of proximal tubule cells is a critically important driving force for solute transport across the brush border and basolateral membranes, it would be anticipated that perturbations in the membrane potential would influence a variety of transport processes. Indeed, in previous studies we have demonstrated that renal cortical brush border membrane Na^+-dependent glucose transport is significantly affected by an increase in relative chloride permeability induced by an acute elevation of tissue cAMP concentration [4]. The present studies confirm and extend this finding by demonstrating that Na^+-dependent glutamine transport is similarly affected (Fig. 3) by the increase in relative chloride permeability that is associated with a chronic elevation in tissue cAMP concentration. It is important to note that the stimulation of these transport processes in membrane vesicles would be expressed as diminished transport in the intact tubule. In vesicles, the increased ionic chloride permeability and imposed inward chloride gradient hyperpolarize the vesicle interior and stimulate electrogenic transport. In contrast, as intracellular chloride concentration is above electrochemical equilibrium in intact proximal tubules [34], the increased ionic chloride permeability and outward chloride gradient would depolarize cells and inhibit electrogenic transport.

It has been estimated that approximately 50% of proximal tubular sodium reabsorption is transcellular with the vast majority driven by the electrochemical gradient for sodium [40]. A reduction in driving force, consequent to cell depolarization, would therefore be expected to diminish the magnitude of electrogenic Na^+-dependent transport processes and thereby diminish transcellular sodium transport. It is of note that electrogenic transport processes are altered in proximal tubule

cells of chronic renal failure kidneys: fractional proximal reabsorption of amino acids [41], phosphate [42], and glucose [43] are decreased. Furthermore, the transepithelial P.D. is decreased (+1.5 to −0.4 mV) in S3 segments of rabbits with renal failure, a finding consistent with an alteration in either cell membrane or tight junction ionic permeability [44]. In the context of the present study, it seems possible that these transcellular electrogenic proximal tubular transport processes may be significantly reduced in renal failure as a consequence of the cAMP associated increase in brush border membrane ionic chloride permeability (Fig. 1). Although not yet examined with electrophysiologic techniques in intact renal proximal tubules, cAMP has been documented to produce marked increases in ionic chloride permeability in other epithelial cells including gallbladder, trachea, shark rectal gland, and renal cortical collecting duct [1–3, 5–8, 31–33]. Of note, cAMP induced increases in chloride permeability result in chloride diffusion potentials of sufficient magnitude to measurably depolarize cells [1, 3, 5]. This effect is particularly striking in that chloride diffusion potentials make insignificant contributions to the basal, resting membrane potential in these tissues [5, 7, 8, 14].

The plasma concentrations of a variety of hormones that act via the second messenger cAMP are chronically increased in renal failure, including parathormone [12], glucagon [12], norepinephrine [13] and antidiuretic hormone [11–13]. Despite the phenomenon of receptor downregulation that occurs in the presence of excess hormone [45], the present study has demonstrated that both renal cortical and red cell cAMP concentrations remain significantly increased in renal failure (Table 3). Although the present studies cannot definitively attribute the increase in membrane ionic chloride permeability to the cascade of events that follow a rise in tissue cAMP concentration in renal failure, a causal relationship is suggested based on the well described effects of acute increments in cAMP on chloride permeability [1–10, 31–33, 39]. While such a causal relationship has yet to be directly established, there are several pathways through which cAMP could effect ionic chloride permeability. Activation of protein kinase A by cAMP could phosphorylate existing chloride channels and increase chloride conductance [6, 31–33]. Since PKA-induced phosphorylation of membrane proteins in vivo induces functional modifications that persist in membrane vesicles [29, 33, 46], the increase in ionic chloride permeability that has been detected in renal failure membranes could reflect such an in vivo event. Of note, a sustained increase in chloride permeability via this mechanism would require that the appropriate phosphatases were not simultaneously activated to dephosphorylate the channels. Alternatively, a cAMP induced increase in intracellular calcium [47] could impact on calcium activated chloride channels [6]. Finally, as cAMP binds to specific promoter regions and activates gene transcription [48], it is possible that the chronic elevation in cell cAMP concentration (at least in renal cells), could enhance transcription, translation and insertion of new chloride channels in the plasma membranes. Any one of these mechanisms could explain the phenomenon of "memory," in which membranes retain their altered properties after isolation from intact cells.

In summary, the present studies have demonstrated that the ionic chloride permeability of renal cortical brush border membranes is increased in association with chronic elevations of intracellular cAMP concentration in a rat model of chronic renal failure. Insofar as this alteration in cell membrane ionic chloride permeability is of sufficient magnitude to influence the membrane potential, it is suggested that this phenomenon may be, in part, responsible for the altered proximal tubule transport that has been observed in chronic renal failure.

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